

CHROMOSOME AND ISOZYME STUDIES IN *TRICHOGRAMMA*
(HYMENOPTERA: TRICHOGRAMMATIDAE)¹

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Abstract.—Karyotype and isozyme analyses were carried out in *Trichogramma chilonis* Ishii, *T. evanescens* Westwood, *T. nubilale* Ertle and Davis, and *T. pretiosum* Riley. No significant differences were detected among karyotypes of these four species as they all have $n = 2SM + 2T + 1A$. However, these species can be readily distinguished on zymograms of superoxide dismutase and esterase.

Trichogramma have been widely used in biological control projects in various parts of the world. However, because of the inadequacy of classical, morphological studies of *Trichogramma*, the precise identities of the species and strains used are in most cases unknown. This problem has been somewhat alleviated by the use of male genitalia as a diagnostic morphological character and crossbreeding experiments as a genetic approach in biosystematics of this group of parasitic insects (see review article by Nagarkatti and Nagaraja, 1977). As pointed out by Harland and Atteck (1933), the basic solution to the problems of systematics in *Trichogramma* is the study of biological characters, crossing relationships, cytology, and genetics of various races and species.

Due to the minute size of *Trichogramma*, cytological and isozyme studies in these insects are rather difficult and have not been adequately carried out. The only cytological study of *Trichogramma* was that of Fukada and Takemura (1943). However, no detailed karyotype analysis was given in their report. Voegelé and Berge (1976) published the first paper on electrophoresis of *Trichogramma*. Further isozyme studies have been carried out by Voegelé and his associates (Jardak et al., 1979; Pintureau et al., 1980;

¹ Approved as TA 21 by the Director of the Guam Agricultural Experiment Station.

Pintureau and Babault, 1981). Their results look promising for use in species differentiation of these difficult parasitic Hymenoptera.

In 1979–80, karyotype and isozyme analyses were carried out at the University of Guam in order to determine the value of these two techniques in the biosystematics of *Trichogramma*. The results of these studies are presented here.

MATERIALS AND METHODS

The following four species of *Trichogramma* were used in these studies: *T. chilonis* Ishii (reared from sphinx moth eggs collected from wild taro leaves on Guam), *T. evanescens* Westwood (from a laboratory culture of W. J. Lewis, USDA, Tifton, Georgia; positive identification of this sample has yet to be determined), *T. nubilale* Ertle and Davis (from a laboratory culture of P. P. Burbutis, University of Delaware, Newark), and *T. pretiosum* Riley (from a laboratory culture of R. K. Morrison, USDA, College Station, Texas). With the exception of *T. evanescens*, species identifications were confirmed by the author. Voucher specimens of these four species are deposited at the Beneficial Insect Introduction Laboratory, IIBIII, USDA, Beltsville, Maryland.

Field collected eggs of a sphinx moth from wild taro were used as host eggs. Freshly laid host eggs were collected in the morning, removed from taro leaves with a camel hair brush, and stored at -10°C in a sealed vial for three weeks in order to kill any egg parasites that might have already parasitized the eggs. After three weeks, the host eggs were removed from the freezer and thawed at room temperature. They were glued in a cluster of ten with Elmer's glue[®] to a strip of filter paper and used for rearing the *Trichogramma* species according to the method of Morrison (1970).

Chromosome preparation.—Due to the minute size of *Trichogramma*, the technique of Hung et al. (1972) was modified. Four to five days after the host eggs were stung, *Trichogramma* pupae with light pink eyes or prepupae were removed from the host eggs and placed on a drop of colchicine-hypotonic solution on a microslide. Testes and ovaries of pupae or brains of prepupae were dissected out and pushed aside to a corner. The remainder of the tissue was then wiped off the slide. The organs to be used were kept on the slide with another drop of colchicine-hypotonic solution for ten minutes. To prevent the organs from drying out, the droplet was covered with a depression slide. After ten minutes, the solution was carefully removed with a piece of filter paper. Special care was used to avoid contact of the filter paper with the organs and at the same time not to dry out the organs. Before the organs dried out, a wax pencil mark was made on the other side of the slide to indicate the position of the organs. A drop of aceto-orcein was then placed on the organs and covered with a cover glass. The materials were squashed between filter paper with the thumb. One drop of immersion

oil was placed on the slide at the position opposite to the wax pencil mark. The pencil mark was then wiped off, and the slide was finally placed under the microscope for examination. Chromosome photographs were taken with Wild M20-EB Phase Microscope® using Fuji Minicopy film®.

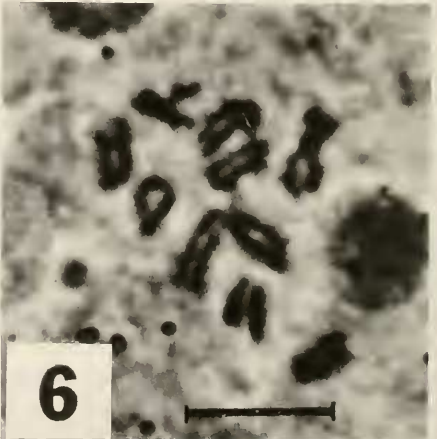
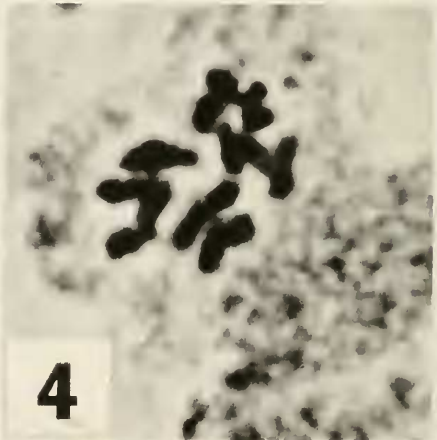
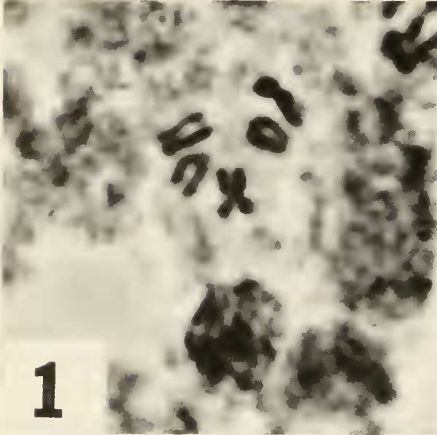
Electrophoresis.—The vertical dual slab cell (Bio-Rad Model 220®) with 0.75×140 mm spacer and Buchler 3-1500® constant power supply were used. The Laemmli system (Laemmli 1970) of reagents and acrylamid gels for SDS electrophoresis was followed in gel preparation, except that in all cases SDS was omitted and sample buffer was replaced with 10% sucrose.

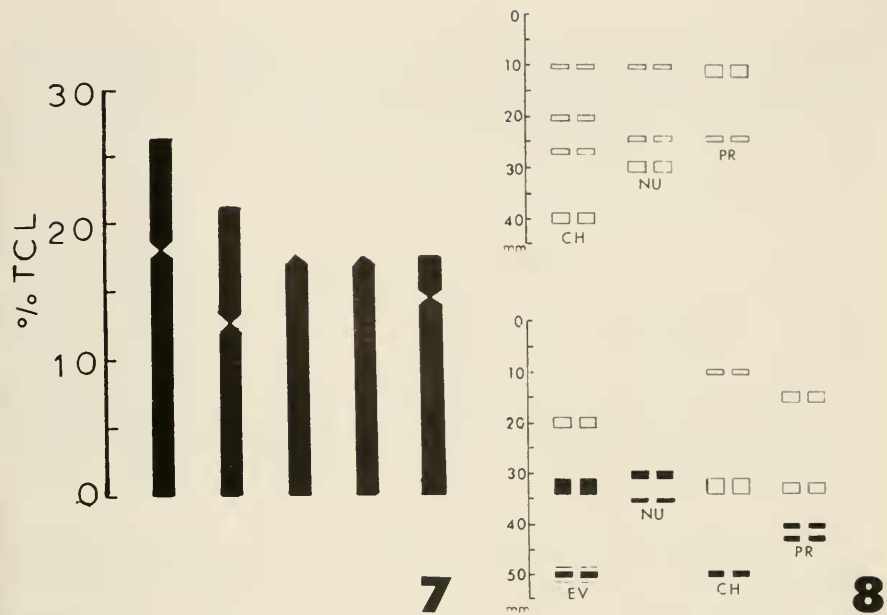
At least five individuals of each species were used for each sample well. The wasps were homogenized in 0.01 ml of 10% sucrose on a 12-cavity white porcelain plate with a glass rod. The homogenate in each cavity was absorbed onto a very narrow strip of Whatman #1 filter paper (ca. 5×0.1 mm). These strips were inserted into the wells already filled with electrode buffer (tris base 6.0 g, glycine 28.8 g q.s. to 1 liter with deionized water). The gel was initially run with constant current of 15 mA until the tracking dye reached the separating gel which took about 3 hours. It was then run for another 2 hours at constant power of 10 watts at which time the gel front would have reached the bottom of the separating gel. During the run, the gel was cooled with circulating ice water. After electrophoresis, the gel was stained for either esterase or superoxide dismutase. The esterase stain consists of 100 ml sodium phosphate buffer, 0.12 M (pH 6.0); 1 ml 1% betanaphthyl acetate (w/v) in 50% acetone-water (v/v); and 40 mg Fast Garnet GBC salt. The stain for superoxide dismutase was composed of 15 mg NADP, 15 mg NBT, 5 mg PMS, 50 mg MgCl₂, and 75 ml 0.2 M Tris-HCl buffer (pH 8.0). All four species were used in esterase assay; however, the culture of *T. evanescens* died later and was thus not included in superoxide dismutase studies.

RESULTS AND DISCUSSION

As shown in Figs. 1–6, these four species of *Trichogramma* each have $n = 5$ which is the same as that reported by Fukada and Takemura (1943) for seven “strains” of *Trichogramma* sp. and is the predominant haploid number in chalcidoids (Crozier, 1977). Further analyses revealed that they not only have the same haploid number, but the morphology of these five chromosomes in each species are the same, i.e. $n = 2SM + 2T + 1A$ as indicated in the idiogram in Fig. 7. This striking homogeneity in the karyotypes of these four species representing two different species groups (Nagarkatti and Nagaraja, 1977) is significant in the application of karyotype analysis in the biosystematics of *Trichogramma*. It indicates that more advanced cytological techniques such as chromosome banding may have to be developed for these minute insects.

Although no significant differences have been detected among karyotypes





Figs. 7, 8. 7, Idiogram based on Figs. 1-6; TCL = total chromosome length. 8, Diagrammatic illustrations of superoxide dismutase (top) and esterase (bottom) banding patterns in *Trichogramma*. CH = *chilonis*; EV = *evanescens*; NU = *nubilale*; PR = *pretiosum*.

of these four *Trichogramma* species, they can be readily distinguished on zymograms of superoxide dismutase and esterase (Fig. 8). Formal progeny analyses to establish the number of loci have not been carried out because at least five wasps have to be used in each well in order to obtain detectable enzyme activity due to the level of sensitivity of slab gel. Nevertheless, the banding patterns of the two enzymes are unique in each of these four species. The esterase zymogram of *T. evanescens* reported here is different from that of Pintureau and Babault (1981). Perhaps my sample is not *evanescens* as I have questioned. Furthermore, whether the three-band phenotype of esterase found in *T. evanescens* represents a heterozygote at this locus has yet to be determined.

A pooled sample of male progeny of a single virgin female has been used to circumvent the difficulty of assaying a single wasp (Pintureau et al., 1980;

Figs. 1-6. Haploid and diploid karyotypes. 1, 2, *Trichogramma chilonis*, male and female. 3, 4, *T. evanescens*, male and female. 5, *T. nubilale*, female. 6, *T. pretiosum*, female. Scale = 10 μ .

Pintureau and Babault, 1981). However, a technique that uses only one wasp per sample well is still highly desirable. This technique would not only facilitate the direct analysis of allele frequency of each population, but also would enable us to use isozyme analysis in hybridization experiments (Hung and Vinson, 1977). Nevertheless, unless chromosome banding pattern turns out to be species specific, isozyme analysis will still be the most useful tool in *Trichogramma* biosystematics.

ACKNOWLEDGMENTS

I am grateful to Dean W. P. Leon Guerrero, Associate Dean R. Muniapan, J. L. Demeterio, Marilou T. Baccay, Sandra L. Dela Garza, and Rosalee Kikuchi for helping me to carry out this study in Guam. I thank P. P. Burbutis, W. J. Lewis, and R. K. Morrison for sending me live cultures and E. E. Grissell and D. R. Smith, Systematic Entomology Laboratory, USDA, for reviewing the manuscript. This study was supported in part by USDA Regional Project W-84.

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